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LIPID EXTRACTION AND TRANSESTERIFICATION TECHNIQUES OF MICROALGAE –A REVIEW

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Abstract: **Microalgae have been identified as a third generation potential biofuel source, which can replace transportation fuels derived from fossil energy sources. Biodiesel production from microalgae biomass is generally performed by one of the two methods: The conventional microalgae biodiesel production essentially involving two main steps; Extraction of oils from the biomass and the oil extraction step involving cell disruption by mechanical, chemical, or biological methods and oil collection by solvent finally, direct in situ production of biodiesel from microalgae biomass. Because vegetable oils or animal fats have high viscosity, i.e., 35–50 $mm^2 s^{-1}$, it is necessary to reduce the viscosity in order to use them in a common diesel engine. Four methods are used to solve this problem: Blending with petro-diesel, pyrolysis, microemusification (co-solvent blending) and transesterification, only the transesterification reaction creates the products commonly known as biodiesel. The in situ offers the advantages of high lipid yield with low loss, simple process with lower cost and time consumption compared to the conventional two steps method.**

Keywords: **Biofuel, Extraction, In situ, Microalgae, Transesterification.**

I. INTRODUCTION

The term biofuel refers to solid (bio-char), liquid (ethanol and biodiesel), or gaseous (biogas, biohydrogen and biosynthetic gas) fuels that are predominantly produced from biomass (Thanh et al. 2012; Kothari and Gujral, 2013). Liquid biofuels are classified into three generations based on the feedstocks (Indhumathi et al. 2014). First generation liquid biofuels were produced from food crops such as corn, sugarcane and vegetable oils (El-shimi et al. 2013; Yadav et al. 2014). Since the food crops are used in the fuel production, first generation liquid biofuels were limited to conflicting with the food supply and increasing the food crop prices (IEA, 2011; Verma et al. 2012; Kumar and Sharma, 2013;).This has paved the way for second generation liquid biofuels, which were produced, using waste cooking oil, non-edible plant seed oil (crops such as jatropha, castor, neem, karanja, rubber seed) (Veera et al. 2013), waste vegetable oil and animal fats (Ei-shimi et al. 2013).

Although second generation liquid biofuels overcame the problems faced by their first generation counterparts, but it there is concern on increasing the fuel consumption and creating a challenge for the supply with consistent feedstock and cost (IEA, 2011; Piasecka et al. 2014) also, they conflict with other commercial products such as cosmetics and industrial products (Veera et al. 2013) this difficulty led to the development of third generation liquid biofuels (El-shimi et al. 2013) like microalgae biodiesel (Kumar and Sharma, 2013b).

Third generation biodiesel feedstock are those that do not conflict with any food, feed or cosmetic related human consumption interests. Work by Veera et al. (2013) reported that macro and microalgae, cyanobacteria, wastewater treatment plant activated sludge, switch grass and other microbial communities belong to this type. Umar, (2014) found that, climate change, greenhouse gas effects, depleting freshwater resources in some regions, growth in human population and shortages of agricultural land will favor the use of third generation biofuel production system such as microalgae. A

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microalga is the term assigned to microscopic organisms often living in colonies. It comprises of both prokaryotic and eukaryotic, mono- and multicellular organisms of sizes from several micrometres to over of dozen metres, they can occur in all fresh and salt, cold and warm waters of all geographical zones (Kumar et al. 2011).

Microalgae can be the source of several types of biofuels such as: methane produced during anaerobic digestion of algae biomass (Anna et al. 2013), straight vegetable oil (SVO), renewable gasoline and jet fuel (IEA, 2011), hydrogen produced photobiologically in anaerobic conditions and biodiesel derived from lipids accumulated as reserve material in microalgae cells (Anna et al. 2013). The desire to have suitable replacement, alternative or an entirely different source of fuel from the presently existing fossil fuel has been very imperative (Highina et al. 2011), as Browne et al. (2010) stated that biofuels derived from algae, however, appear to be the most feasible at present date. This paper present an overview on microalgae used for biodiesel production including the extraction techniques, use of catalyst, and processing. Another objective is to present a detail direct in situ transesterification process in biodiesel production and the option of two step conventional method.

II. BIODIESEL PRODUCTION PROCESSES

Microalgae biodiesel production essentially involves two main steps:

- Extraction of oils from the biomass (Martinez-guerra et al. 2014), and the oil extraction step includes cell disruption by mechanical, chemical, or biological methods and oil collection by solvent (Park et al. 2014).
- Conversion (transesterification) of oils (fatty acids) to biodiesel (alkyl esters) (Martinez-guerra et al. 2014)

To date, biodiesel production from algae biomass is generally performed by one of the following methods:

(1) A two-step protocol in which algae oil is extracted and then converted to biodiesel using a catalyst, such as an acid, a base, or an enzyme.

(2) Direct single step in situ production of biodiesel from algae biomass using an acid catalyst at atmospheric pressure and ambient temperature.

The current microalgae-based biodiesel is mainly produced by conventional route: extraction of the lipids from the microalgal biomass followed by its conversion to FAMEs and glycerol. However such method is time consuming, costly, and difficult to be implemented in algae's crushing steps because of the rigid cell walls (Li et al. 2011).

Some researchers that work using the in situ method (Chattip et al. 2010; Marck et al. 2012; Gulab et al. 2012; Shah et al. 2012; Nguyen et al. 2013) reported that in situ transesterification differs from the conventional reaction in that the oil-bearing material contacts with alcohol directly instead of reacting with pre-extracted oil and alcohol also, compared with the two step method, this process increase FAEE yield with highly purified extracts, bypassing the use of large quantities of organic solvents, reduced energy consumption and easier purification steps.

III. TRANSESTERIFICATION

Transesterification is the reaction process to change the triglyceride molecules present in animal fats or oils reacts with an alcohol. Indhumathi et al. (2014) stated that the purpose of the transesterification process is to lower the viscosity of the oil. The reaction is often catalyzed by acid and base catalyst or enzyme (Utoma, 2013). Anna et al. (2013) found that biodiesel could be methylic or ethylic esters of fatty acids; it is synthesized in the process of transesterification of TCG with methanol or ethanol. Transesterification is an equilibrium reaction in which excess alcohol is required to drive the reaction close to completion (Park et al. 2014).

Biodiesel is defined as the mono-alkyl esters of fatty acids (FAMEs) (Li et al. 2011) derived from vegetable oils or animal fats (Patil, 2011). When a vegetable oil or animal fat is chemically reacted with an alcohol in a presence of catalyst such as sodium or potassium hydroxide, it produces a new compound that is known as the fatty acid alkyl ester. According to Patil (2011), the approximate proportions of the reaction are:

100 kg of oil + 10 kg of methanol →100 kg of biodiesel + 10 kg of glycerol (1)

Because vegetable oils or animal fats have high viscosity, i.e., 35–50 mm^2 s^{-1}, it is necessary to reduce the viscosity in order to use them in a common diesel engine (Thanh et al. 2012) they also found that four methods are used to solve this problem: Blending with petro-diesel, pyrolysis, microemusification (co-solvent blending) and transesterification and further stated that among these methods, only the transesterification reaction creates the products commonly known as biodiesel.

Biodiesel is usually produced by transesterification of lipids (oil) of oleaginous plants such as soya bean (Malakootian et al. 2014), corn (El-shimi et al. 2013), palm, rapeseed, sunflower, jatropha (Rahman et al. 2010), neem (Kumar and Sharma, 2013b) pongamia (http://www.oilgae.com/extract/extract.html), cottonseed (Veillette et al. 2011) and organic oils (Kumar et al. 2011). In fact, biodiesel is derived from the transesterification of mono-, di- and tri-acylglycerides (TAGs) and by transesterification of virtually any triglyceride feedstock (Azean and Yilmaz, 2012).

3.1 Direct Transesterification (In -situ):

Direct in situ transesterification or "reactive extraction" is a process that combines the steps of lipid (oil) extraction and transesterification to produce biodiesel thereby avoiding the steps of cell disruption and oil extraction from biomass and it includes both the esterification of free fatty acid and the transestrification of triglyceride from microalgae (Park et al. 2011; Umar, 2014). The block diagram of single step is presented in figure1.

Brian and Zheting, (2014) experimented a method that involves the simultaneous addition of the acid catalyst and pure methanol to microalgal biomass (generally in the form of dried powder). El-shimi et al. (2013) make similar findings and discovered that the methanol extracts the lipids from the microalgal biomass an, catalyzed by the acid, concurrently transesterifies the extracted lipids to produce fatty acid methyl esters. Work by Li et al. (2011) and Utomo, (2013) discovered that, recently the single step transestrification method has received serious attention for algae-based biodiesel production.

3.1.1 Advantages of Single Step In Situ:

✓ Integration of extraction and transestrification stages could minimize biodiesel production cost, since the use of reagents and solvents is reduced and the analysis is easier and not expensive (Li et al. 2011; El-shimi et al. 2013; Choi et al. 2014).

✓ Combining the oil extraction step with transesterification improves the economics of overall biodiesel production.The insitu transesterification method has the potential to simplify the conversion process, reducing the number of unit operations and consequently the overall process costs and final biodiesel product costs (Martinez-guerra et al. 2014).

✓ The advantages of the in situ transesterification include the simplified process for converting microalgal lipids to algal biodiesel and the reduction of some technological challenges encountered in traditional processes (Brian and Zheting, 2014).

✓ This process simplifies the production process and improves the biodiesel yield compared with conventional extraction because of the elimination of oil extraction step that incurs oil loss (Park et al. 2014).

✓ The direct derivation methods can result in greater FAME yields than are achieved in the two step extraction (Salama et al. 2013; Selvakumar and Umadevi, 2014).

✓ The direct method has shown to be effective in making biodiesel from both pure and mixed cultures of microganisms (Johnson and Wen, 2009).

✓ This process could reduce the long process time and also maximize biodiesel yield as well as use of reagents and solvents, waste water production and significantly maximized ester yield (Li et al. 2011; Utomo, 2013; Choi et al. 2014)

✓ Consumes less amount of solvent compared to traditional transesterification (Umar, 2014).

3.1.2 Limitations of Single Step In Situ:

For direct transesterification, a high level of methanol and sulfuric acid is required compared with a commercial biodiesel process (Kumar and Sharma, 2013a). One of the drawbacks of this process is that, final oil extraction via this process consumes 2 times more energy than the oil extraction process via the transesterification (Umar, 2014) he further explain

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that Fatty acid retention in solid lipids removes the difficulty with lipid recovery from the system which ideally consumes energy, expensive and sometimes hazardous.

Choi et al. (2014) and Park et al. (2014) discovered that the overall process of directly producing biodiesel (FAME) from fresh microalgae has not been well investigated and did not identify even key parameters in employing in situ transester-ification for microalgae that contain relatively hard and rigid cell membranes, which require longer process time and larger extraction solvents, etc.

Dewatering and drying of algae is necessary to reduce the water content to 5% before the oil extraction process (Pardo-cardenas et al. 2013). However, current papers provide limited information about both quality and quantity studies of in situ transesterification from microalgal biomass therefore, further research is needed to develop a feasible method for high yield and high quality in situ biodiesel production from microalgae (Malakootian et al. 2014) as conducted by Salama et al. (2013).

3.2 Two Step Conventional Process Transesterification:

Biodiesel (FAME) can be prepared from algal biomass through conventional method first; oil is extraction from algal biomass followed by transesterification (Li et al. 2014). In the conventional process, the recovery of green crude from microalgae generally requires multiple solid-liquid separation steps. These processes involve drying, cell wall disruption, and solvent extraction. The extraction of green crude is usually performed with toxic organic solvents such as hexane, chloroform, and methanols, meaning these processes are highly energy-intensive and environmentally damaging. In the lab-scale, green crude extraction with hexane normally carried out by soxhlet at 70 °C for 18 hours. This long time of extraction and heating is drawback in the hexane extraction method.

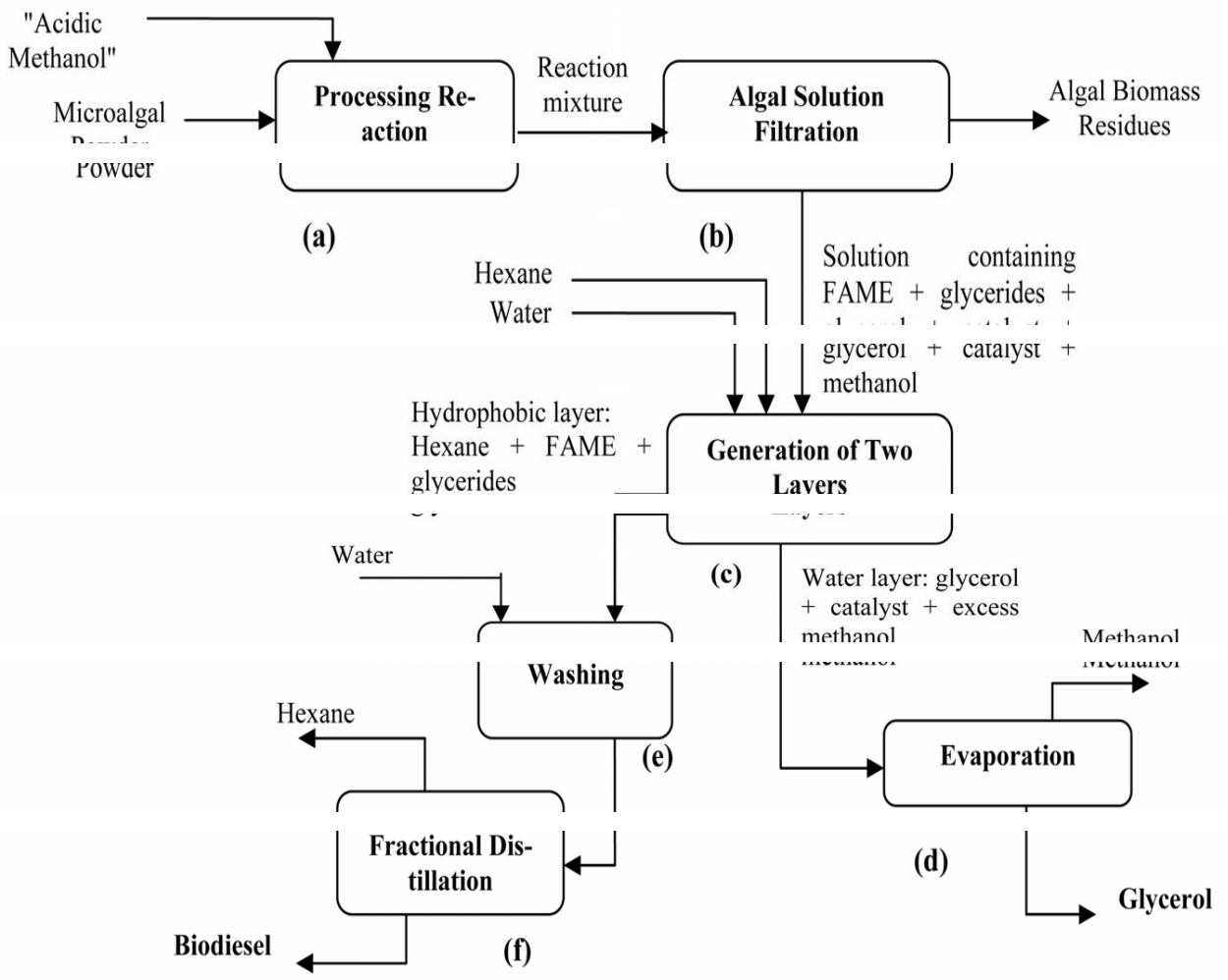


Fig. 1: Block diagram of the in situ transesterification steps used for biodiesel production (Sources: El-shimi et al. 2013).

The most rapid and effective conventional extraction method for green crude is the Bligh-Dyer's method,as experimented by Selvakumar and Umadevi (2014) who uses drying, cell disruption, solvent (chloroform-methanol) extraction, and evaporation of the solvent. This method has been indispensable and standard, not only for green crude extraction from micro algae but also the quantification of the crude oil from biological materials (Kanda and Li, 2011).

The conventional methods for lipid extraction generally involve dewatering before extracting lipids since residual water in wet microalgal biomass hindered mass transfer of the lipids from the cell and then lead to a decrease in the efficiency of lipids extraction. Further dewatering of algae cells can be achieve by amassing the cells in to filterable solids prior to transesterification, this may as well save cost (Umar, 2014).

The energy consumption in drying accounted for the majority of the total process energy (84.9%) as reported by Yang et al. (2014), In addition, the organic solvents used in the conventional methods are regarded as highly-toxic, being environmentally unfriendly. These shortcomings hinder the application of conventional methods in industrial lipid extraction, despite of the high extraction efficiency.

Table I: Comparison of Extraction-transesterification and in situ methods (Li et al. 2014)

Insitu Transesterification	**Extraction-Transesterification**
Heating value is high	Heating value is low
Yield is high	Yield is low
Very simple processes in operation	Process is complex
Due to absence of extraction and dewatering the production cost is low	Production cost is less
Avoided the potential lipid loss	lipid loss during process
Reduced the waste water pollutants	waste water pollutes environment
Less time consuming process	Time consumption is high

3.3 Catalysts:

TCG transesterification reaction may undergo catalysis in different ways: by the use of acids, bases or enzymes (Rudy, 2012; Anna et al. 2013) and Supercritical methanol process (Patil, 2011; Rudy, 2012). Only recently, transesterification process optimization has been found to be possible with microwaves application (Anna et al. 2013). Catalyst plays significant role in the up gradation through transesterification of bio-oil to biodiesel. According to Yadav and Varma (2014), both the lipid/oil extraction from algal biomass and its up gradation to biodiesel are relatively new concept and system is not well understood.

3.3.1 Alkaline Catalysts:

Homogeneous alkaline catalysis has been the most used route for biodiesel production because it catalyzes the reaction at low temperature and atmospheric pressure and high conversion yield can be achieved in a short time (Azean and Yilmaz, 2012). Sodium hydroxide (NaOH) and potassium hydroxide (KOH) are widely used as alkaline catalysts (Park et al. 2014; IEA, 2011). These catalysts operate by reacting with the alcohol according to the reaction given below (written using methanol and NaOH but other alcohols and catalysts could be substituted).

$$CH3OH + NaOH \rightarrow CH3O\text{-}Na + H2O \qquad (2)$$

However, alkaline catalysts cause the free fatty acids in oils to produce soap and are not suitable for microalgal biodiesel production because of the high free fatty acid content in microalgal oils.

Research by Xiaochen, (2012) showed that under the high concentration of alkaline catalyst, KOH will react with fatty acid and form emulsion formation between soaps and water molecules. As the catalyst concentration increased, more soap

will be produced which consume the catalyst and reduces the catalytic efficiency. When the high concentration KOH catalyzed, crude biodiesel was purified by washing with sodium chloride solution, the water phase solution is cloudier.

Saponification will also bring other problems, like increases in viscosity, the formation of gels, and difficulty in achieving separation of glycerol. Removal of these saponified catalysts is technically difficult and it adds extra cost to the production of biodiesel (Thanh et al. 2012). Most of the biodiesel produced today is done with the base catalyzed reaction as observed by Patil (2012) or with Supercritical Methanol in comparison with acid catalyzed esterification hence, Highina (2010) found that the based-catalysed transesterification is much faster, and less corrosive, than the acid catalyst.

Basic catalysis is the highest-speed transesterification method (it arrives 4000 times faster than acidic catalysis with the use of the same amount of catalysts), however, it can be applied only in the case of water-free oils, otherwise saponification occurs and newly formed soaps decrease catalysis effectiveness and disturb glycerol separation from post reaction mixture. Recently basic transesterification method was optimised enabling single transesterification stage (Anna et al. 2013).

3.3.2 Acid Catalyst:

The most used acid catalysts are sulfuric acid (H_2SO4) and hydrochloric acid (HCl) (Thanh et al. 2012). They require larger response times and higher temperature than alkaline. In some studies, initially, an acid catalyst is used to convert free fatty acid into esters through esterification, after the free fatty acid content in the oils is reduced to less than 1%, a second transesterification step for the oils is performed by using an alkaline catalyst (Park et al. 2014). Acidic catalysis is slow with longer reaction time in addition to causing undesired corrosion of the equipment, but it is mostly suitable for transesterification of oils with water and high content of free fatty acids (Anna et al. 2014).

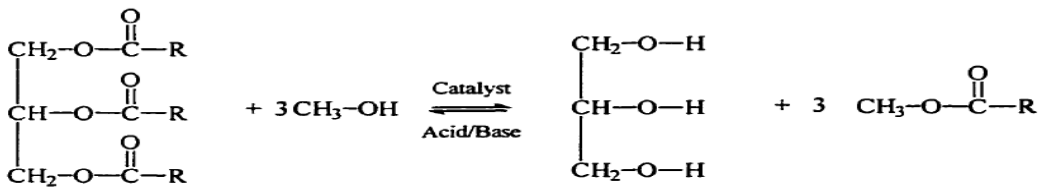
Research work by Pardo-cardenas (2013) have shown that as biodiesel is obtained by means of the transesterification of the TAG's of the oils, there are some cases in which a high content of FFA's makes the oil too acid. This fact makes the basic transesterification no longer the best alternative, to cope with this, a process has been developed so that before the basic transesterification takes place, there is an esterification of the FFA's with an alcohol in the presence of an acid catalyst. As a result, fatty acid alkyl esters and water are obtained and separated, at this point; an alkali-transesterification of the TAG's with alcohol will take place faster and with less technical difficulties.

3.3.3 Enzymatic Catalysts:

Enzymatic catalysis with the use of lipases is also possible (Azean and Yilmaz, 2012) however, its costs are very high (Thanh et al. 2012). According to Highina (2010), lipase catalyzed transesterification is carried out in nonaqueous environment since chemical transesterification is efficient in terms of reaction time more so, the chemical approach to synthesize biodiesel from triglyceride has drawbacks such as difficulty in the recovery of glycerol and the energy intensive nature of the process. In contrast, biocatalyst allows synthesis of specific alkyl esters usually recovery of glycerol and transesterification of glycerides with high free fatty acid content.

3.4 Alcohols for Transesterification:

Different types of alcohols can be used to produce biodiesel such as methanol, ethanol propanol and butanol or amyl alcohol (Anna et al. 2013; Choi et al. 2014). Nevertherless, methanol and ethanol are the most widely used in due to lower cost biodiesel (Utomo, 2013) both in the laboratory research and industrial process (Jin et al. 2014). Transesterification needs 3 alcohol molecules for every TCG to produce 3 molecules of methyl esters (Kumar and Sharma, 2013a).



Triglyceride (oil) Methanol Glycerol Mixture of methyl esters

To achieve 95% efficacy of esters generation, the reaction is performed in alcohol significant excess (Anna et al. 2013).

3.4.1 Methanol:

Methanol is most often produced out of natural gas or coal so, in contrast to ethyl ones, methyl esters cannot be fully derived from renewable energy sources. It is mainly derived from petroleum-based sources and is associated with toxic properties (Jin et al. 2014). The majority of the world methanol production is based on natural gas (Martinez-guerra et al. 2014), it has a low boiling point and excess from the glycerol phase is easily recovered after phase separation. Currently, methanol is a commonly used reagent for transesterification and acts both as extraction solvent and esterification reagent (Park et al. 2014).

Generally, methanol plays the role of both reactant and extrant in in situ transestrification (Salama et al. 2013; Selvakumar and Umadevi, 2014), but it was proved to be a poor solvent for oil extraction, this would probably lead to low conversion efficiency from algal biomass to biodiesel by in situ transestrification (Li et al. 2011). The amount of methanol is one of the most important factor affecting quality and quantity of biodiesel production. An exceeding amount is essential for in situ biodiesel production moreover; the excess of methanol quantities also slows down the separation process (Li et al. 2011).

The need to recover methanol from the products streams may add additional cost to the process as reported by Salama et al. (2013). In their work, they further stated that the amount of methanol and sulfuric acid should be reduced to avoid the need for a large reactor and reactor corrosion by sulfuric acid. Solvents such as pentane and diethyl ether have been used to reduce the volume of methanol by enhancing the reaction yield.

The higher toxicity of methanol relative to ethanol also raises additional concerns regarding its transportation and storage (Martinez-guerra et al. 2014). Despite safety issues, methanol is preferred on industrial scale because of its high reactivity and low cost (Pardo-cardenas et al. 2013).

3.4.2 Ethanol:

Ethanol is attractive for long term sustainability since it can be derived from renewable sources, it is less toxic than methanol, and generally considered safe (Martinez-guerra et al. 2014). The result of Yang et al. (2014) investigation implied that ethanol had potential for extracting lipids from wet microalgae at room temperature. Furthermore, the work confirmed that the recycled ethanol had high efficiency for extracting lipids from wet biomass therefore; the extraction method with ethanol was suitable for extracting lipids from microalgae at large scale, with a high extraction efficiency and low environment pollution. The lipids arising from microalgal biomass by ethanol, referred to as crude lipids, frequently contain several non-lipids (proteins bonding to lipids strongly and carbohydrates) (Patil, 2011; Yang et al. 2014).

Ethanol is less toxic than methanol and renewably derived, it has superior dissolving power for oils, and produces biodiesel with improved fuel properties (e.g higher energy density, larger, cetane number, and stronger oxidation stability) (Jin et al. 2014).

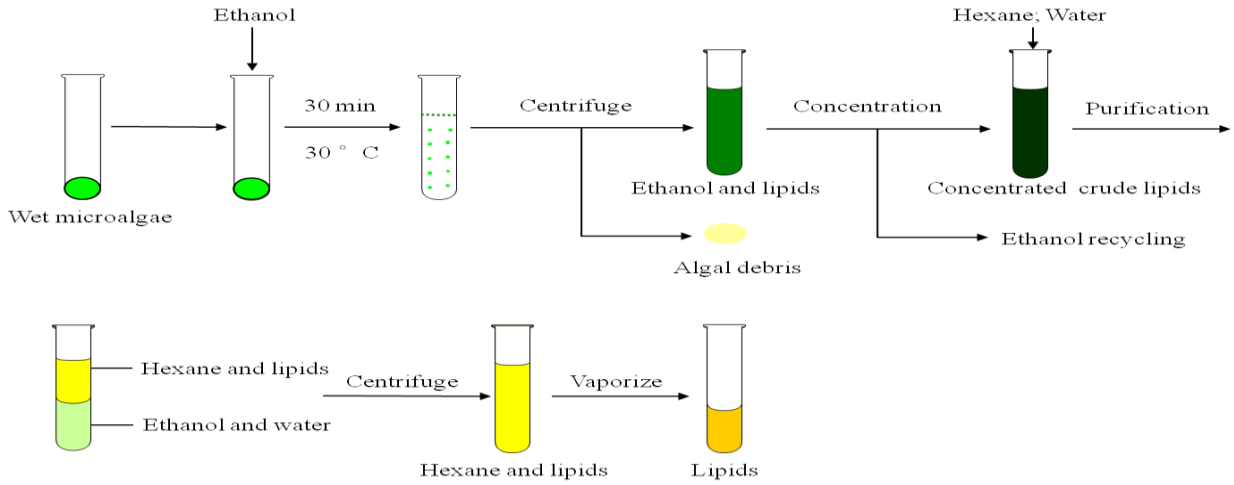


Fig.2: A scheme illustration of lipid extraction procedure from wet microalga using ethanol. (Source: Yang et al. 2014)

3.5 Solvents and Extraction Techniques:

Two types of extraction are wet extract processes that focus on disrupting the algae cells in solution, and dewatering methods which remove the algae from aqueous water solution and then mechanically or chemically disrupt the cells (Browne et al. 2010). Niraj et al. (2011) used green algae and hexane (solvent extraction method) to recover almost all the oil to leave behind only 0.5% to 0.7% residual oil in the raw material. Extracting lipids is one of the most key and limited processes for biofuel production based on microalgae at large scale.

According to Li et al. (2011), addition of solvents such as n-hexane or chloroform to the in situ reaction system could lead to a higher biodiesel yield. Furthermore, their work suggested that in direct transestrification, nonpolarity solvent (cyclohexane) should be a better choice than polarity ones (hexane). The addition of hexane could help oil extraction so that half of the methanol could be saved (Li et al. 2011). The use of an additional solvent such as hexane or chloroform helps the easy extraction of oils within microalgae cells and enhances the contact of its oil with the esterification reagent (Selvakumar and Umadevi, 2014).

A wide variety of organic solvents are often used to extract oil from microalgae, where hexane and ethanol is the most common as reported by Pardo-cardenas et al. (2013). However, ethanol is a polar solvent and its selectivity to lipids is relatively low compared to other solvents, so in extractions with ethanol, other microalgae components may also appear, such as sugars, pigments or amino acids. Using a hexane-ethanol mixture, approximately 80% of fatty acids present in biomass can be extracted. Hexane is cheaper than other non-polar solvents such as cyclohexane; it is easy-to-recover after extraction and is selective to neutral lipids. In addition, it can be used in mixture with isopropanol (Pardo-cardenas et al. 2013).

In general, solvent mixtures containing a polar and a non-polar solvent could extract a greater amount of lipids. For example, a combination of chloroform (non-polar), methanol (polar) and water, known as the Bligh & Dyer method, has been used for lipid extraction from a wide range of biological samples (Veera et al. 2013).

3.5.1 Microalgae Cell Disruption:

Extracted microalgae oils are typically converted to biodiesel by transesterification using alcohols and catalysts. The oil extraction yield of dry microalgae cells is relatively higher than that of wet microalgae cells. During the oil extraction process, internal oils are excreted into the outside medium by disruption of thick cell walls and the oils are partitioned into hydrophobic solvents such as hexane which is a low-toxicity solvent employed to remove non-lipids complex from crude lipids (Yang et al. 2014).

Lipid extraction from microalgae biomass is done through various means: mechanical method (Kumar and Sharma, 2013a) through: expression/expeller, microwave /ultrasonic-assisted extraction (Niraj et al. 2011; Azean and Yilmaz, 2012), organic solvent by: hexane solvent method, soxhlet extraction, supercritical fluid extraction (Ben et al. 2010; Rudy, 2012; Li et al. 2014), and enzymatic extraction (Utomo, 2013). Kothari and Gujral (2013), found that Non-mechanical techniques include processes such as freezing, acid, base, and enzyme reactions, use of organic solvents, and osmotic shock to name a few. In spite of recently developed extraction methods including supercritical fluid extraction or microwave/ultrasound assisted extraction, lipid extraction from biomass by organic solvents was still found superior in comparison to others because of simplicity, relatively low cost and the non-requirement for special equipment (Malakootian et al. 2014).

Each of these methods has drawbacks: The mechanical press generally requires drying of the algae, which is an energy intensive step; the use of chemical solvents poses safety and health issues; however, solvent extraction is usually applied to get high oil yields from algae; supercritical extraction requires high pressure equipment that is both expensive and energy intensive (http//www.oilgae.com/extract/extract.html).

IV. ECONOMIC CONSIDERATIONS OF BIODIESEL PRODUCTION

Production costs of biodiesel from microalgae are even higher when compared to conventional fuels as observed (Christi 2007; Suali and Sarbatly 2012; Surendhiran and Vijay, 2013) that producing microalgae biomass is generally very expensive compared to the final yield of the product yet, microalgae remains imperative as future energy feedstock.. There are few studies about it due to the novelty of the process as such, the biodiesel from microalgae biotechnology

production parameters remain to be optimized in order to decrease the production cost (Veillette et al. 2011). All stages involved in microalgae biodiesel production chain require high energy, thus contributing to high production costs (Pardo-cardenas et al. 2013). According to Xu et al. (2006), biodiesel usually costs over US$0.5L^{-1} compared to US$0.35L^{-1} for conventional diesel fuel. Some attempts have been made to reduce the cost such as using cheap carbon sources for high oil microalgae production example, Jerusalem artichoke, cassava starch, and sugar cane juice were introduced as new carbon supplies for microalgae cultivation and algae-based biodiesel production (Li et al. 2011).

Report by Thanh et al. (2012) discloses that more than 10 million tons of biodiesel fuel (BDF) have been produced in the world from the transesterification of vegetable oil with methanol by using acid catalysts (sulfuric acid, H_2SO4), alkaline catalysts (sodium hydroxide, NaOH or potassium hydroxide, KOH), solid catalysts and enzymes. Unfortunately, the price of BDF is still more expensive than that of petro diesel fuel due to the lack of suitable raw material oil.

To access the potential of biodiesel as substitute of diesel fuel, its properties such as density, viscosity, flash point, cold filter plugging point, density, solidifying point, and heating value need to be determined (Xu et al. 2006; Umar, 2014). These parameters must comply with the limits established by ASTM related to biodiesel quality. Economic impacts of biodiesel are sustainability**,** increased number of rural manufacturing jobs, increased farmer income and agricultural development.

V. CONCLUSION

Microalgae appear to be the source of renewable biodiesel that is capable of meeting the global energy demands, since energy is essential for economic development of the world and global economy runs on energy. The first and second generation biofuels are produced from various food crops and plant seed oils. microalgae has been identified as a third generation potential biofuel source, which can replace transportation fuels derived from fossil energy sources due to various advantages than the previous two generation feed stocks.

The extraction of lipid from microalgae can be achieved by expeller or press, solvent extraction with organic solvents, or supercritical fluid extraction. After extracting the oil from microalgae, transesterification process is mostly adopted for synthesis of biodiesel. There are several transesterification methods to produce biodiesiel such as: two steps conventional method, in situ process, ultrasound assisted process, microwave, and enzymic. Release of solvent in extraction-transesterification process contributes to the production of atmospheric smog and to global warming and is classified as a hazardous air pollutant. In situ transesterification method is a promising method which reduces the disadvantages of extraction-transesterification method. It offers the advantages of high lipid yield with low loss, simple process with lower cost and time consumption compared to the conventional two steps method. Different types of alcohols can be used to produce biodiesel such as methanol, ethanol propanol and butanol or amyl alcohol. Nevertherless, methanol and ethanol are the most widely used in biodiesel due to lower costs both in the laboratory research and industrial process. Finally, to obtain lipids from microalgae, the overall process of choosing an algal strain, cultivation, harvesting, dewatering, and extraction of oil are quite complicated.

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